AMEBAS OF WARM MINERAL SPRINGS, FLORIDA, INCLUDING *Cochliopodium papyrum* N. SP.[1]

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About twelve miles southeast of Venice, Florida, and three-fourths of a mile east of U. S. Highway 41, a warm brackish spring of major size, known as Warm Salt Springs (Ferguson, et al., 1947), and commercially as Warm Mineral Springs, empties by way of a two-mile-long creek into the Myakka River near the southern border of Sarasota County. The rapid rate of flow, (seven to nine million gallons of water per day) a constant temperature of 86° F., and an unusual concentration of salts present a formidable habitat for microorganisms. Ferguson, et al. (1947), Odum (1953), and Lackey (1957) have published data indicating the dissolved mineral content to be about half that of sea water in total dissolved solids (17,998 ppm. as compared to 34,482 ppm.), chloride (9,350/18,980 ppm.), sodium (4,973/10,556 ppm.), and magnesium 567/1,272 ppm.). The spring contains about two-fifths the potassium found in sea water (151/384 ppm.); but one-third more calcium (596/400 ppm.), six times more silica (24/4 ppm.), and four and one-half times more iron (0.09/0.02 ppm.). Sulfate content is about two-thirds that of sea water (1,704/2,639 ppm.). There is a total phosphorus content of 0.050 ppm., and an inorganic phosphorus component of 0.033 ppm. (Odum loc. cit.). Lackey (loc. cit.) reports there is no dissolved oxygen. He records 0.162 ppm. of hydrogen sulfide; a slightly alkaline pH of 7.2; and a chemical oxygen demand of 813 ppm. Thus, while approximately half as "salty" as sea water, the salt content is different in balance, and presents difficult problems of osmoregulation to any animalcule immersed in it.

Lackey (loc. cit.) cites ciliates as a numerous fauna, though of an unusual association of species. He further states that amebas and flagellates are "poorly represented". He implies either a paucity of different species or a limited number of individuals, or both, though he does not say. He mentions as flagellates one green

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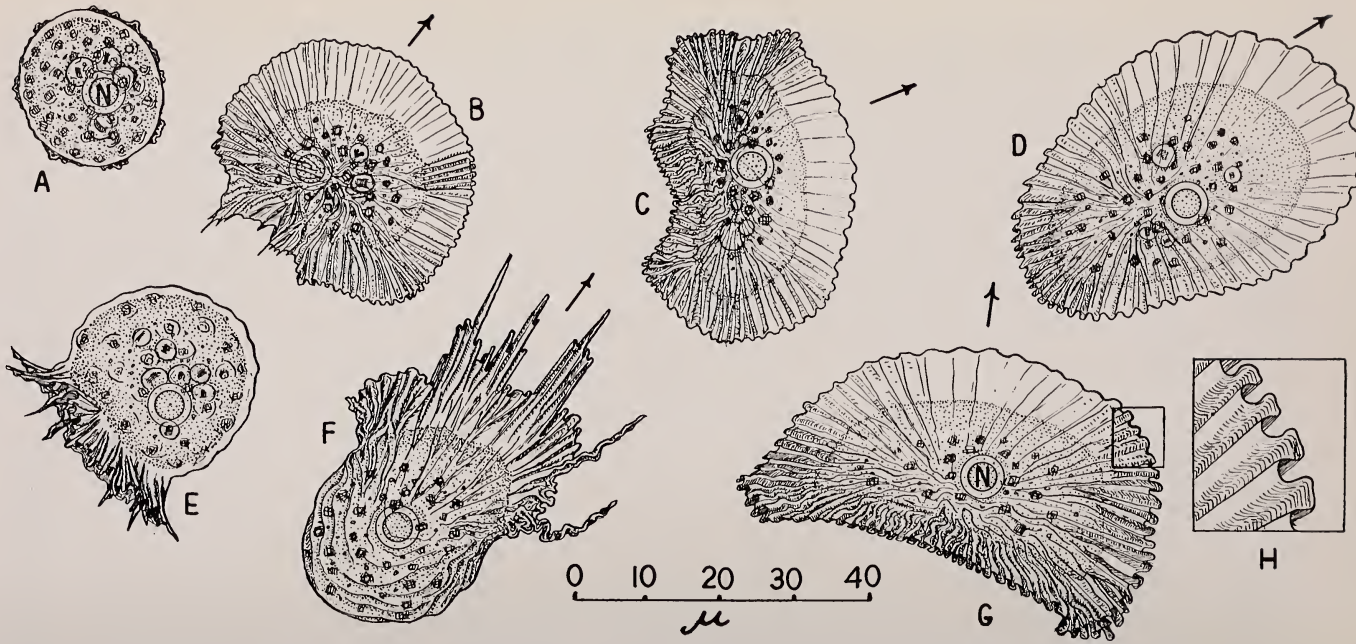

Figure 1. (A) *Cochliopodium papyrum* n. sp., inactive. (B) In slow locomotion, showing the typical corrugated folds of the clear border. (C) In moderately rapid locomotion, beginning a turn to its left, as evidenced by the pronounced corrugations at the left border. (D) In moderately rapid advance just after a turn, the ameba moving in the direction of its long axis. (E) A floating individual, seen laterally, and showing the much contracted and folded clear border. (F) Same individual as in (E), just after descent from afloat and during slow movement just prior to locomotion. The folded pseudopodal extensions of the clear wave are temporary. (G) *Cochliopodium papyrum* n. sp. in very rapid progress, fan-shaped and with the upper surface much wrinkled. (H) An enlargement of the area outlined in (G), showing in detail the folding of ectoplasm composing the clear, bordering wave. (N) is the nucleus; other spherical inclusions being food vacuoles. The irregular inclusions are the amorphous crystals. There is no contractile vacuole. Arrows indicate direction of movement.

and several colorless euglenoids. He names no amebas, though implying their presence.

Three water samples taken from Warm Mineral Springs on December 28, 1957, were examined repeatedly by brightfield and interferometer microscopy during a period of two weeks thereafter. Five species of amebas, known previously from marine waters, were found to have invaded and currently inhabit the springs. Three of these were named and carefully described by Schaeffer (1926) in his monograph. They are: *Flabellula mira; Flabellula* (Rugipes) *vivax*; and *Trichamoeba sphaerarum*. A fourth ameba is morphologically identical with *Vexillifera telmathalassa* (Bovee, 1956).

The fifth was tentatively identified as belonging to the genus *Hyalodiscus* Hertwig and Lesser (Bovee, 1958 a and b), but later comparisons clearly place it in the genus *Cochliopodium* Hertwig and Lesser (1874). A search of the literature indicates that it is a new species. It will be hereinafter described.

In two of the water samples, amebas were scarce; but in the third, the species named above were present in abundance in an algal-sulfur bacteria-ciliate association taken where the overflow of the main spring flows into the creek.

Though only five species of amebas have been found in the Warm Mineral Springs, large numbers of individuals may be present in some localized, ecological associations. The scarcity of amebas mentioned by Lackey (loc. cit.) seems to refer principally to the number of species more than to the number of individuals.

It is evident, also, that the species of amebas which live in the spring are physiologically tough and adaptive, capable of extensive osmoregulation, and already so, probably, before entering the spring. *Flabellula mira* is an ubiquitous animal, found in marine waters (Schaeffer, 1926), in brackish and fresh waters (Bovee, 1953 and 1958a), and able to survive transfer to distilled water or to sea water ten times concentrated by evaporation (Hopkins, 1938). *Flabellula* (Rugipes) *vivax* occurs naturally in a variety of marine and brackish water situations (Bovee, 1958a).

Vexillifera telmathalassa withstands changes in salt concentrations due to evaporation and rainfall, and the daily temperature changes in exposed tide pools. A very similar species occurs nat-

urally in fresh waters a few miles inland from the sea (Bovee, 1950 and 1958b).

Trichamoeba sphaerarum is strictly marine according to Schaeffer (1926), dying quickly in sea water diluted 50% with distilled water. The morphologically identical organism in the Warm Mineral Springs must therefore have undergone considerable adaptation.

How the amebas originally entered the spring is not known, but the frequent presence of gulls and crows at the spring suggests that the amebas may have survived transfer in mud on the feet of these birds. Immigration upstream from the sea is also possible, but seems less likely. Subsurface transfer in underground seepage is unlikely since soundings of the spring indicate more than 167 feet of depth (Ferguson et al., 1947).

Cochliopodium papyrum n. sp.

General contours: The ameba is ovate to subspherical at rest, with a thick ectoplasm resembling a pellicle, the surface of which is somewhat wrinkled and papulate. In slow movement the ovate body flattens, and the pellicle extends as a wrinkled skirt-like periphery about half the width of the central mass. In beginning locomotion the clear border at one "side" is extended as a series of crinkled, conical, clear pseudopods. As locomotive speed increases, the body becomes ovoid and the clear border more pleated. At greatest speed of advance, the body is fan-shaped anteriorly and almost straight to slightly concave at the rear border, the upper surface and clear border being much wrinkled, resembling a crinkled mass of paper. No radiate stage was seen.

Size: At rest the ameba measures 17 to 22 μ in long diameter, 14 to 18 μ in short diameter, and 10 to 7 μ in vertical diameter. During slow movement the body mass measures 15 to 20 μ in diameter, and the total diameter across the body mass and surrounding clear wave, 35 to 40 μ. In slow locomotion the posteriorly placed, body mass is subspherical, 25 to 32 μ in horizontal diameter, the anterior, clear border being 7 to 10 μ in extent, and the pseudopods 4 to 25 μ long, overall length 50 to 60 μ. While the ameba is in moderate locomotion, the body mass measures 25 to 35 μ in width, and 15 to 25 μ in length, the surrounding border being 7 to 10 μ wide, and the overall length 35 to 40 μ, with overall width being 44 to 55 μ. The central body mass during rapid advance is

10 to 15 μ measured antero-posteriorly, and 24 to 28 μ measured laterally, the surrounding clear wave extends 7 to 10 μ anteriorly and laterally, and 4 to 6 μ posteriorly. The overall length is 20 to 25 μ and overall width 37 to 45 μ.

Color: Practically colorless, this organism borrows the colors of its ingested food, which is mainly plant detritus, so that the body mass may appear to contain brownish or yellowish droplets, greenish fragments, and many pale yellowish-green amorphously-crystalline inclusions. The protoplasm is nearly colorless with perhaps a slight bluish tint when observed by brightfield microscopy, and is a pale reddish purple by interferometric dark-contrast phase microscopy.

Locomotor organelles: Pseudopods are not regularly formed by this ameba. Only in slow locomotion do extensions of protoplasm resembling pseudopods appear. These are extensions of the anterior margin of the surrounding clear wave which are 3 to 25 μ long, tapering, conical, crinkled and folded lengthwise, being almost pointed at the tips, and 3 to 6 μ wide at their bases. When the organism is disturbed and goes afloat similar pseudopodal structures may develop.

In more rapid locomotion the clear wave is generated by outflow of protoplasm midway along the anterior border, being displaced laterally by more outflow. The laterally pushed elements of the wave fold in linear corrugations from body mass to the edge of the wave. These fold more and more tightly together until they reach the posterior border of the ameba. There the parallel folds crinkle and contract along their lengths, and meld back into the general body mass.

Rate of Locomotion: This ameba is torpid much of the time, and slow in its movements when active. When disturbed it may go afloat, with the ectoplasmic wave much folded and crinkled about the previously ventral portion of the body. Upon descent to the substrate it remains inactive for an hour or more before beginning to move about, even under rather intense illumination. In its most rapid progress along a rather sinuous path, it advances only about half its antero-posterior extent in one minute, about 20 μ. It is most active in mid-day hours, very rarely active in early morning or late afternoon hours, moving slowly amongst bacterial floc and algae on the bottom of the stream.

Uroidal Structures: These semi-permanent, posterior, gelated structures or tufts of filaments do not develop in the activities of this ameba.

Surface Markings: At rest, and during slow movement and locomotion, amorphous crystalline inclusions rest just under the thick pellicular membrane, causing it to bulge slightly over each crystal so that the surface appears somewhat papulated. In more rapid movements, the clear margin is pleated and the dorsal surface of the body mass thrown into wrinkles and folds which are more or less continuous with the marginal folds.

Nucleus: This organelle is usually approximately central in the body mass. It is pale and difficult to see clearly. Light passing through it is not strongly and differentially refracted, so that the nuclear membrane is not clearly discerned; that of the nuclear endosome being more distinct. The nucleus is 6.5 μ in diameter, spherical in form. The endosome is central within the nucleus, also spherical, very pale, but with a slight bluish tinge under brightfield microscopy, and pale bluish-purple under interferometer-phase microscopy, measuring 4 μ in diameter.

Crystals and Granules: About two dozen irregularly crystalline particles from less than one micron to about 3 μ in diameter are present, located mostly in the gelated protoplasm just beneath the thick, pellicular ectoplasm which covers the body mass. These appear pale yellowish-green under brightfield microscopy, and dark purple with yellowish "highlights" under interferometer, dark-contrast phase microscopy. Also present are many tiny granules less than 0.5 μ in diameter which give the endoplasm a pale bluish tint under brightfield and a reddish-purple color under dark-contrast phase.

Food Vacuoles: Several of these are usually visible, containing bits of vegetable detritus in various states of digestion. The vacuoles vary in size from about one micron in diameter to about 5 μ in diameter, greenish to orange in color under brightfield and yellowish to reddish-orange under phase microscopy. They form beneath the body mass as the ameba glides over the food particle.

Contractile Vacuoles: None are present.

Habitat: A brackish spring, 86° F., Warm Mineral Springs, Sarasota County, Florida.

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